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(54) Chromatography cartridge

(57) A chromatographic method and apparatus for purifying proteins generally, and monoclonal antibodies from ascites fluids specifically, is disclosed. The method comprises contacting a solution containing proteins to be purified with a porous, substantially nonswellable sheet which has hydrated crystalline hydroxylapatite particles physically immobilized therein, in the presence of a pH buffer, and then eluting purified protein fractions, such that the flow rate and back pressure do not change significantly throughout the method. One preferred apparatus comprises inlet and outlet seals (10, 15), spiral-wound or core-wrapped chromatographic separation medium (60) comprising hydrated crystalline hydroxylapatite particles physically immobilized in a porous, substantially nonswellable sheet, and a porous central support (40) upon which the sheet is wrapped. Another apparatus comprises single or stacked chromatographic separation medium comprising hydrated crystalline hydroxylapatite particles physically immobilized in a porous, substantially nonswellable sheet.

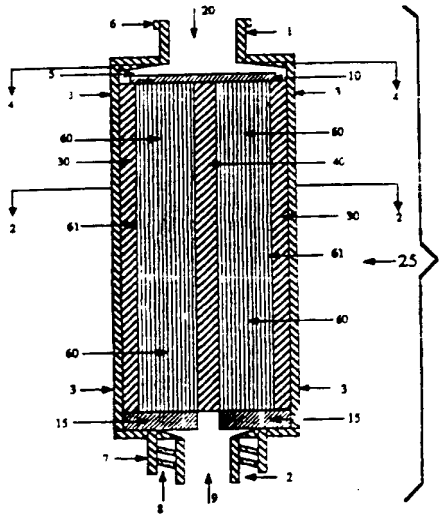
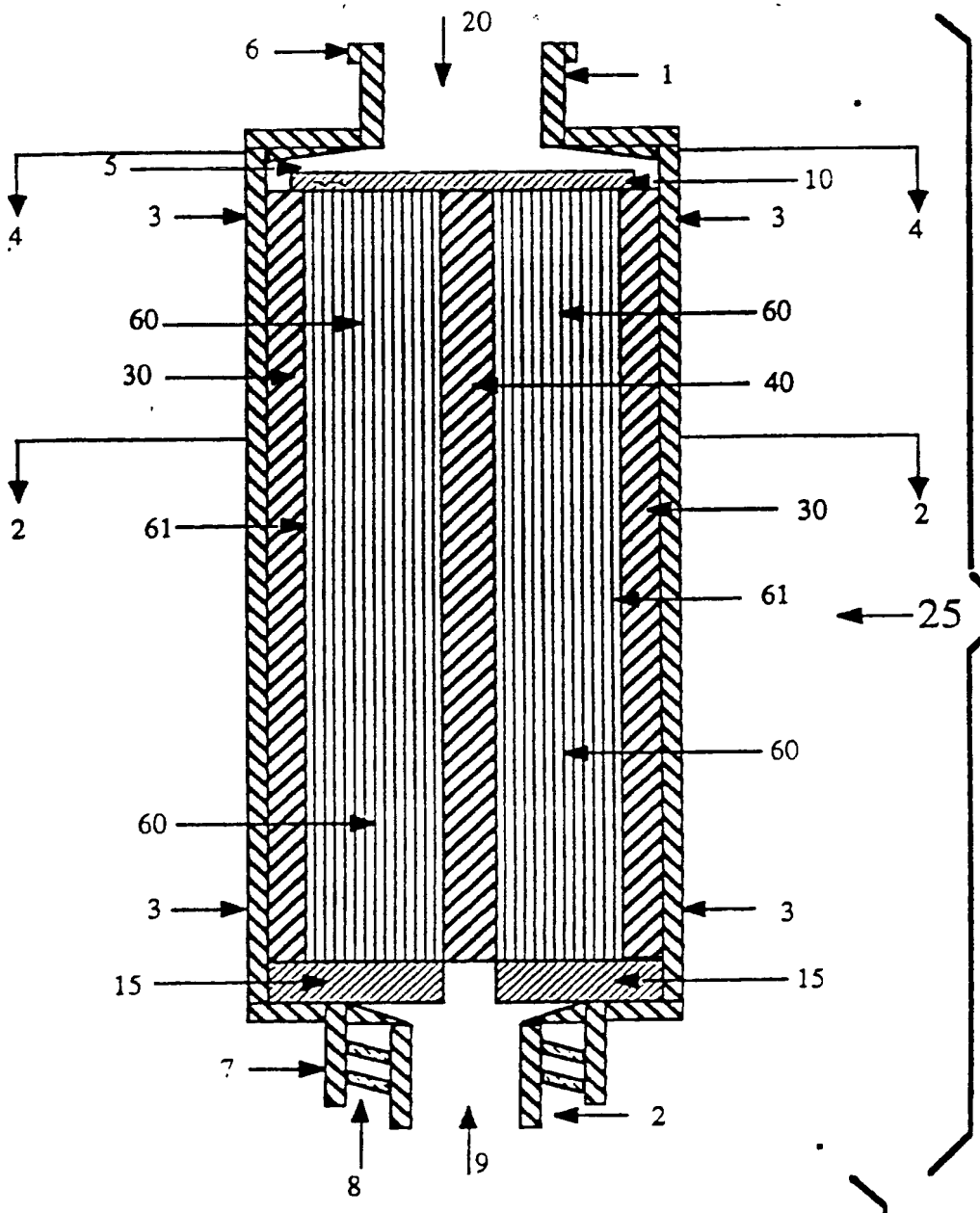


FIG.1

GB 2 246 716 A


20
6
1
5
10
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3
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4
60
60
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40
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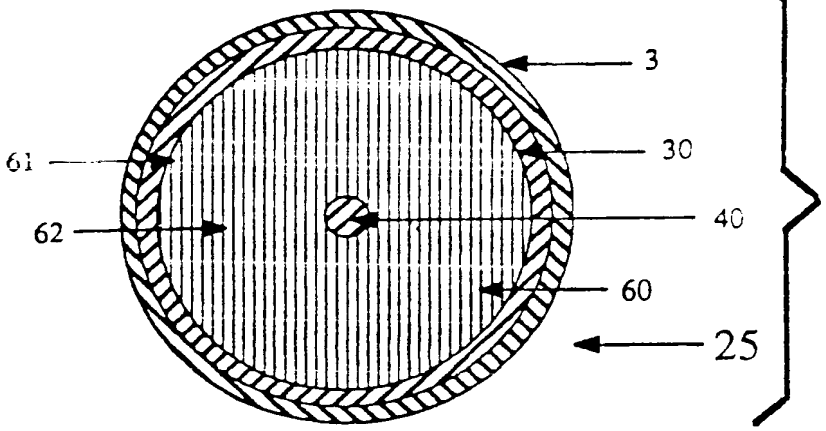
25
60
60
3
3
15
15
7
2
8
9

FIG.1


3
61
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62
40
60
25

FIG.2

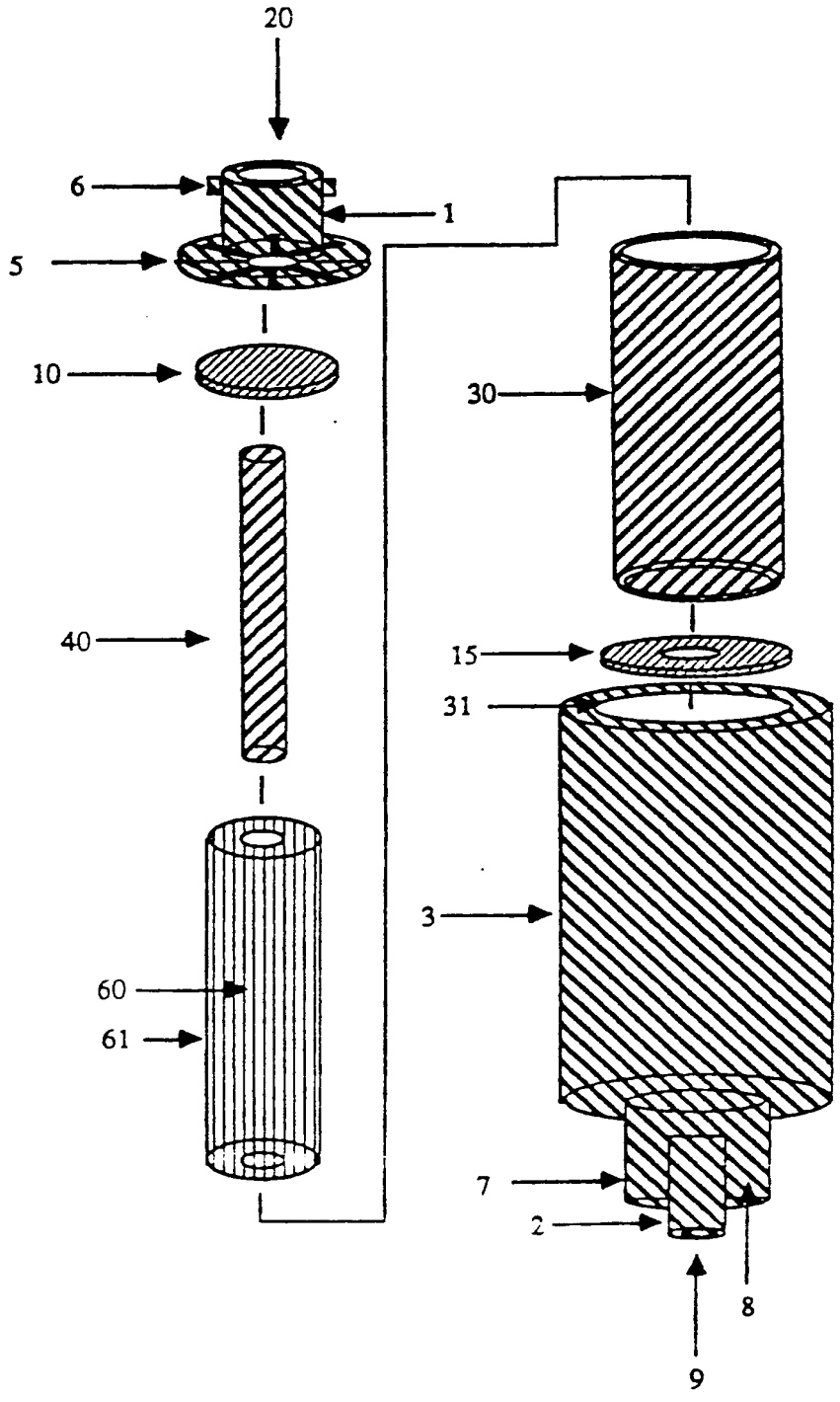

FIG.3

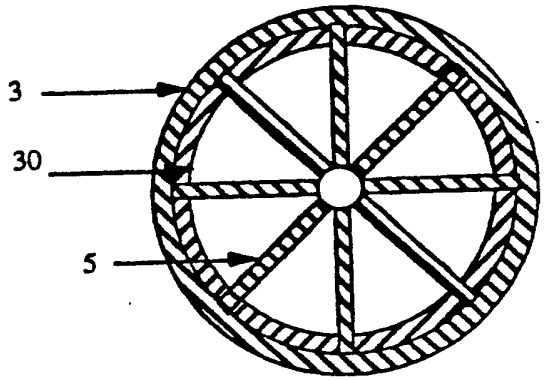

FIG.4

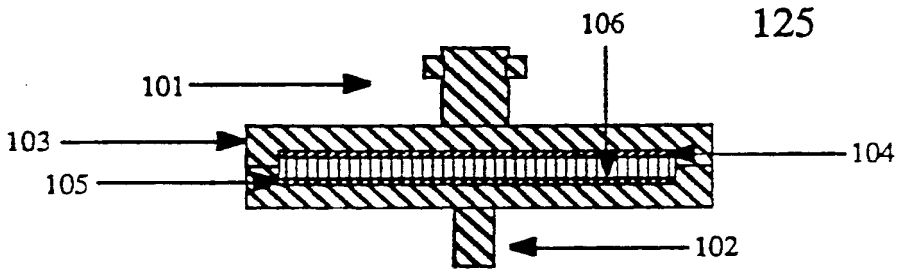

FIG.5

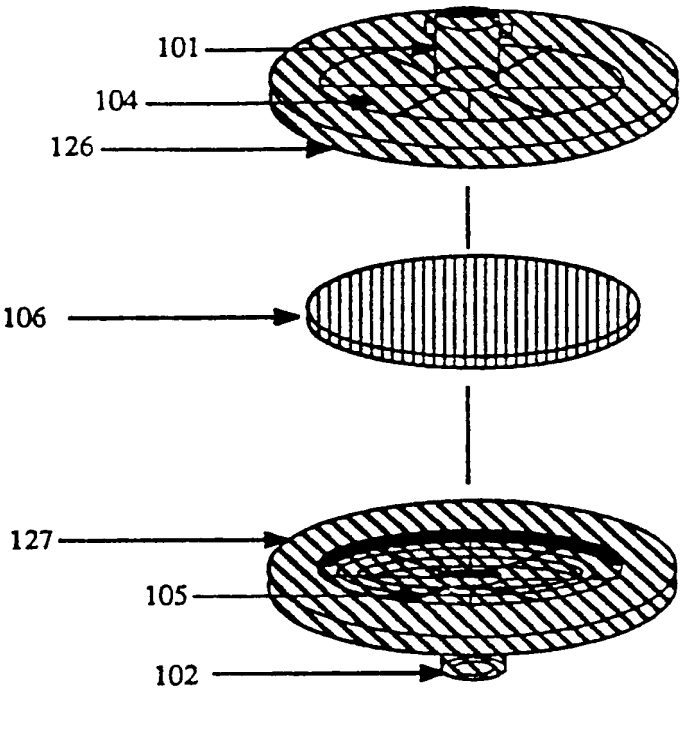

FIG.6

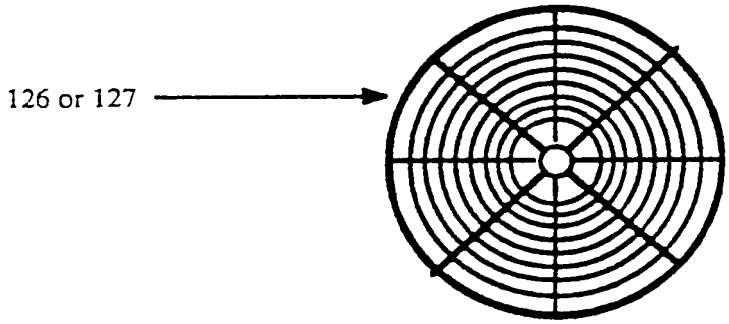

FIG.7

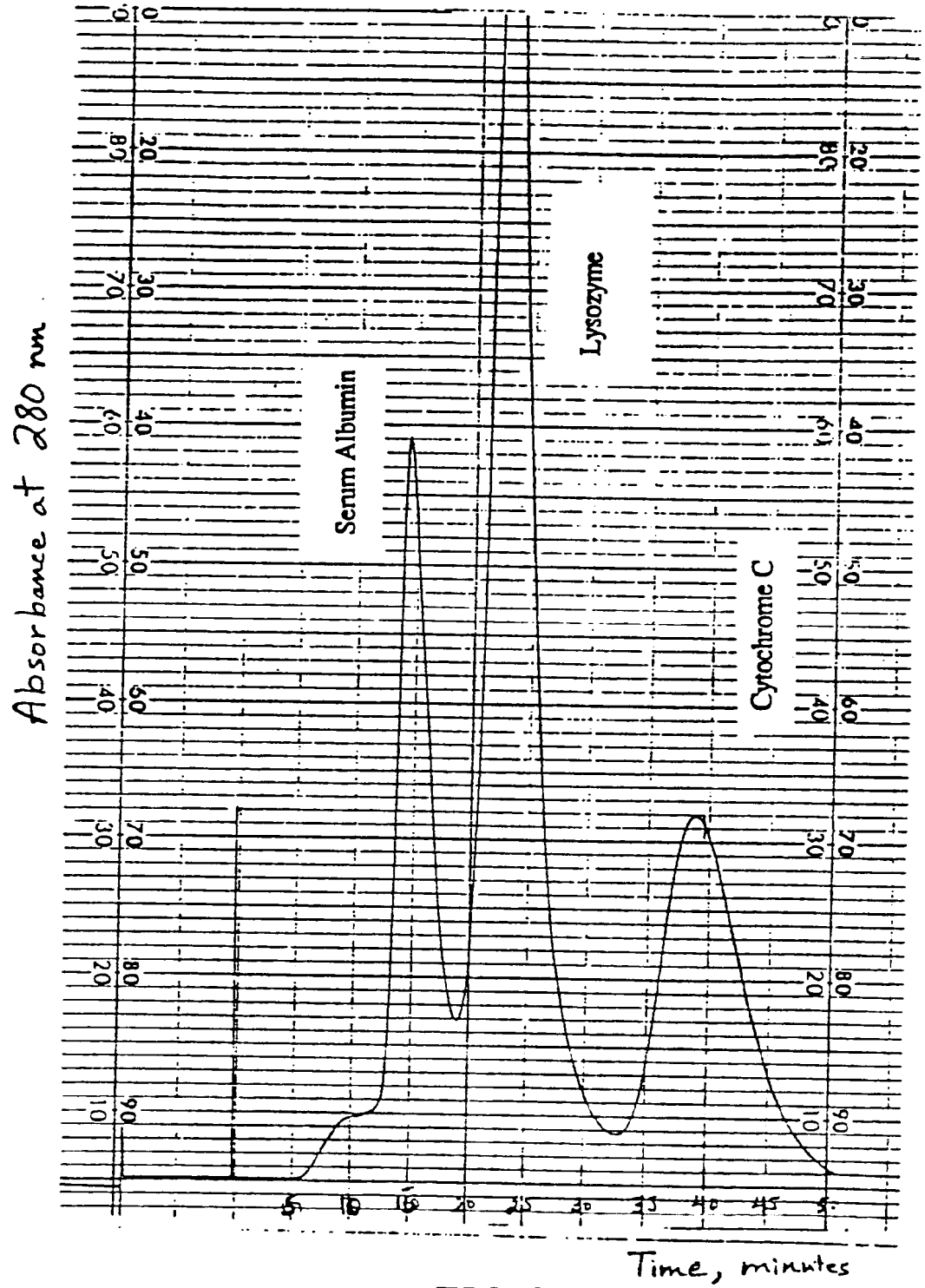
Absorbance at 280 nm
Serum Albumin
Lysozyme
Cytochrome C
Time, minutes

FIG.8

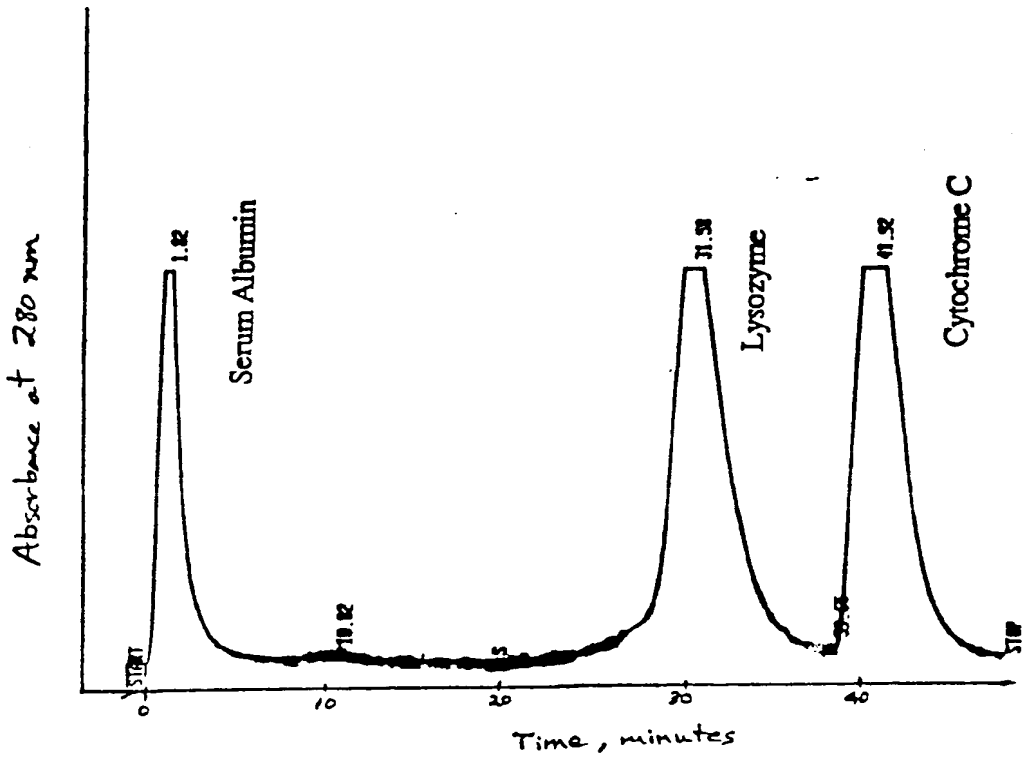
Absorbance at 280 nm
Serum Albumin
1.82
Lysozyme
11.58
Cytochrome C
11.92
START
0
10
20
30
40
Time, minutes

Fig. 9

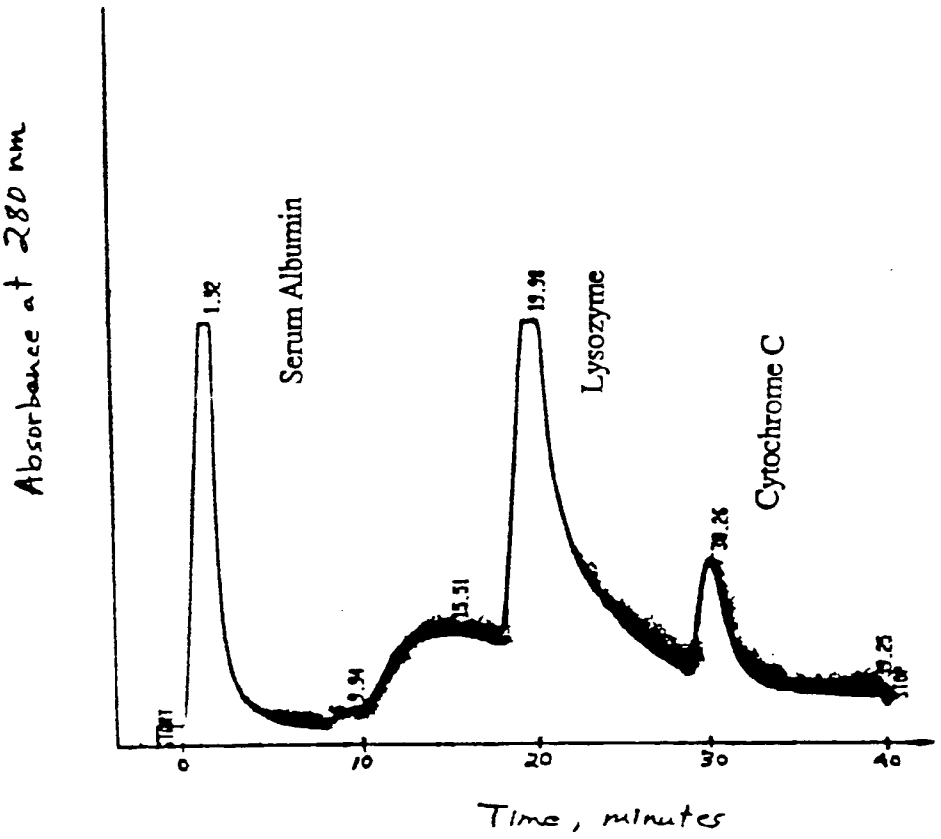
Absorbance at 280 nm
Serum Albumin
Lysozyme
Cytochrome C
1.92
15.51
15.98
20.26
0
10
20
30
40
Time, minutes

Fig. 10

CHROMATOGRAPHY CARTRIDGE

This invention relates generally to methods of separation of biological materials. Specifically, this invention relates to a chromatographic method and apparatus for purifying large quantities of antibodies in a quick and efficient manner.

In the art of purifying complex proteins such as polyclonal and monoclonal antibodies, the focus has been on the ease and speed of obtaining purified antibody solutions. In a very general description, one may characterize one method of purification of these substances from ascites fluid as a three step chromatographic process. The first step may be applying ascites fluid to an adsorption chromatography column in which hydroxylapatite (HA) particles are packed. This material, which is similar to and may be identical to the complex salt of which bone is comprised, has a special ability to adsorb proteins, while allowing other biological and nonbiological molecules to pass through. This step separates complex and simple proteins from lipids, fats, salts, nucleotides and polysaccharides.

The second step may be to further purify antibody molecules such as IgG's by an ion-exchange process. Ion-exchange chromatography takes advantage of the charges on proteins to bind them to beads of a charged support medium such as DEAE or QAE bound to Sephadex particles. In anion-exchange chromatography, proteins are applied at a basic pH such as 8.6 at which they are either negatively charged (albumin, alpha-1, alpha-2, and beta globulins (such as transferrin) are anions) or have no net charge (gamma globulins, also referred to as immunoglobulins). The neutral proteins pass immediately through an anion exchange column matrix. By passing through buffer with a higher salt concentration, anions of the salt displace the support medium allowing the proteins to elute from

the column. By utilizing a steadily increasing gradient of salt concentration in the eluting buffer, the complex proteins can be even further purified according to charge. However, further purification is often necessary.

If the protein of interest has a specific binding propensity such as an immunoglobulin for an antigen, a third step may be affinity chromatography. This is a very powerful tool for selecting from all of the immunoglobulins in a sample only the specific ones that bind to the antigen which is covalently coupled to the support medium. After washing off all the proteins which do not adhere, the high affinity material is eluted with a very high salt concentration or some chemical denaturant such as urea.

Immunoglobulins can also be adsorbed using staphylococcal protein A coupled to Sepharose. For example, United States Patent No. 4,704,366 to Juarez-Salinas and Ott, assigned to Bio-Rad Laboratories, Inc., Richmond, California, discloses unusually strong binding of IgG to protein A achieved by contacting these components in the presence of a medium containing a high concentration of salt. The patentees state that a particularly useful application is the purification of monoclonal antibodies from ascites fluid by affinity chromatography. In this way, specific cuts of subclasses of immunoglobulins may be prepared, for example IgG_1, IgG_{2A} and IgG_{2B}. This method is also known as "hydrophobic interaction chromatography."

As stated above, the focus of the prior art has been on improvements in speed and ease of purifying and/or concentrating monoclonal antibodies. Automated procedures have been developed, using the latest software and hardware technology. In the first step, separation of proteins from other biological and nonbiological material, advances have been achieved in reducing the amount of pressure needed to force greater amounts of sample solutions through columns packed with HA. Bukovsky and Kennett (Hybridoma, Vol. 6, No. 2, 1987) describe a method of simple and rapid purification of monoclonal antibodies from cell culture supernatants and ascites fluids by HA adsorption chromatography, on analytical

and preparative scales. They disclose use of high flow Bio-Gel™ HT (Bio-Rad) rather than the fine powder DNA-grade HA. The DNA-grade HA is claimed to produce too small flow rate to be useful for processing large volumes of sample. Also, a combination of step-wise and linear gradient phosphate elutions result in substantially increased purity of the monoclonal antibodies.

The procedure of Bukovsky and Kennett is said to have two advantages over other HA adsorption chromatography systems. First, the method is said to achieve separation with a low back pressure requirement, requiring only a peristaltic pump on a preparative scale column, with a flow rate of 2 to 3 milliliters per minute. Back pressures of 40 to 60 PSI at the rate 0.5 to 0.8 milliliters per minute on analytical scale columns are also disclosed. Second, the method uses relatively high flow rates, so that 500 milliliters of cell culture fluid can be purified in eight hours. Bukovsky and Kennett disclose a thirty fold monoclonal antibody concentration by using a phosphate gradient elution followed by step-wise phosphate concentration elution.

Although the methods of Bukovsky and Kennett are indeed impressive, even higher flow rates and lower back pressures are desirable. It would especially desirable if higher flow rates and lower back pressures could be achieved without development of new mobile phases. Further, it is desirable to have chromatography column solid stationary supports which can be reused many times and retain essentially the same flow and pressure characteristics. Conventional column packing of HA allows "freshly poured" Bio-Gel™ HT columns to achieve high flow at low back pressures initially, but after six or seven runs the column packing must be replaced by the chromatographer, as the flow rate decreases substantially with a concurrent increase in pressure drop. This is due to the nature of hydroxylapatite itself: the hydrated form has the formula $Ca_{10}(PO_4)_6(OH)_2$. As the crystalline particles are hydrated in the conventional column packing configuration, they expand slightly. In conventional packed columns this causes rubbing of adjacent particles. When

the hydrated crystalline particles are exposed to a typical phosphate buffer solution, the phosphate ion causes compaction of the HA bed. This expansion and compaction are deleterious to the bed, causing plugging by "fines." This usually entails removing the old HA column cartridge and replacing with a new cartridge, which sacrifices time due to temporarily taking one cartridge off line.

One method developed to date to solve a similar plugging problem in ion-exchange chromatography is the use of "volume compensation frits" in the cartridges. This technique is described in U.S. Patent No. 4,871,463, issued to Taylor and Rogler-Brown, assigned to Sepratech, Carlsbad, California. The patentees describe the use of volume compensating frits which expand and contract as the packed ion-exchange resin particles change volume on exposure to various pH and ionic strength solutions. The patent also claims use of a flow distributor which distributes flow across the entire cross-section of the solid stationary phase and a flow collector which collects flow across the entire cross-section of the solid stationary phase.

Composite sheets have been developed, made of various materials including polytetrafluoroethylene (PTFE) in which various particulate matter has been permanently enmeshed. For example, in United States Patent 4,460,642, to Errede, et al., assigned to Minnesota Mining and Manufacturing Company, St. Paul, Minnesota, the inventors disclose water swellable composite sheets comprising PTFE fibrils in which particulates, such as Bio-Rad cation exchange resins, are permanently enmeshed. The patentees disclose that these composite sheets are useful for gas phase or liquid phase chromatography because of their porous nature and very uniform distribution of substrate which prevents "channeling," but does not, however, prevent the spiral flow between membranes unless intermembrane spaces are minimized. The adsorptive particles are thoroughly enmeshed in the PTFE fibrils so that substantially all of the particles are contained in the fibrils and do not slough in the wet or dry state. PTFE is presented at the surface of the composite sheets. The adsorptive particles are not on the surface of the composite but are strongly enmeshed in the tough

PTFE fibrils. There is thus little chance of any of the particulate matter to slough off. The PTFE fibrillated surface is not rendered adhesive by other materials because it is claimed to be nonadsorptive and nonwetted due to its unusually low surface tension, despite the fact that composites of these materials can be very hydrophilic. The patentees do not disclose use of HA. However, the patentees do disclose that in highly water swellable composite sheets the composite sheets are stiffer and less flexible than those which swell less. They suggest addition of up to 70 weight percent as a percentage of particulate, of non or slightly water swellable particulates, to provide greater flexibility and softness to such composites while retaining good strength. Examples of "diluent" particles disclosed include kaolin, talc, silica, bentonite, and vermiculite. The patentees describe these "diluents" as inert, having no effect on a chromatographic separation.

In the art of making composite sheets, glass, polymeric, and cellulose fiber silica-composite sheets are made using paper making technology. These fibrous papers are used in common filtration applications. Ahlstrom Filtration, Inc., Mt. Holly Springs, Pennsylvania, produces such composite sheets by methods known in the art. The composite sheets can be made from several types of inert fibers and absorptive particles to 98 percent retention. Composite sheets can contain between 40 and 90 weight percent adsorptive particles while retaining good dry strength. Wet strength is poor unless binders are added to the composite sheet. HA has not been used in the composite sheets as absorptive particles.

In the search for methods of obtaining uniform flow across preparative size columns, composite sheets have been used in a spiral-wound configuration, (U.S. Patent No. 4,743,373 to Rai, et al.). This patent describes a "swellable matrix in sheet form" with "spacer means between each layer" of the spiral-wound sheets for permitting controlled swelling of the swellable matrix. This can be classified as a variation in structure of the "volume compensation frits" of the later issued Taylor/Rogler-Brown patent, described above. Rai, et

al., describe their solid stationary phase as providing even distribution of sample flow without an increase in pressure drop when compared to a stationary phase not utilizing the spacers.

To date there has not been developed a system of quickly separating proteins from ascites fluids without the use of volume compensation frits or spacer means between porous, substantially nonswellable, spiral-wound or core wrapped, or single or multiple stacked chromatographic media, with no change in flow rate or pressure drop, using HA adsorption techniques.

It has now been discovered that high, constant capacity HA adsorption chromatographic separation of proteins from other molecules can be achieved using crystalline HA particles permanently enmeshed in a porous, substantially nonswellable sheet, without the use of, or with only residual, volume compensation. As used herein the term "substantially nonswellable sheet" means that the sheets are efficienty packed tight in the cartridges used herein in a dry condition, then slightly swell to conform to the inner contours of the cartridge. The sheets may then compact slightly back to their original dry, efficiently packed state, when contacted with buffer solutions described herein.

One advantage of this format is the great reduction in back pressure required to force samples through the cartridge. Typically, only a syringe is need. Another advantage is that rather than six or seven runs per cartridge, as with conventional packed bed columns, the use of HA particles permanently enmeshed in a material such as polytetrafluoroethylene (PTFE) or glass fibers allows tens of runs per cartridge. Also, the nonwettable surface properties of PTFE allows the entire surface of a composite sheet to be exposed to incoming flow of sample in a chromatographic device, thereby enhancing flow capacity to a point where liters of sample can be processed in a fraction of the time of conventional HA packed bed columns. And perhaps most

importantly, the porous, substantially nonswellable sheet allows the HA particles to hydrate and dehydrate in such a way that no fines are produced, and the particle surface area is held constant for contact with incoming sample containing monoclonal or polyclonal antibodies.

Further improvements, advantages, embodiments and aspects of the invention will become apparent from the description which follows by way of example only, with particular reference to the drawings, in which:-

FIG. 1 is a partial sectional view of a side elevation of one embodiment of the chromatography cartridge in accordance with the invention;

FIG. 2 is an enlarged cross-sectional view taken along line 2-2 of FIG. 1;

FIG. 3 is an exploded perspective view of all of the parts of the chromatography cartridge shown in FIG. 1;

FIG. 4 is an enlarged cross-sectional view taken along the line 4-4 of FIG. 1;

FIG. 5 is a partial sectional view of a side elevation of another embodiment of the chromatography cartridge in accordance with the invention;

FIG. 6 is an exploded perspective view of all the parts of the chromatography cartridge shown in FIG. 5;

FIG. 7 is a view of the flow distribution or flow collection grid impressed on the inner surfaces of the chromatography cartridge shown in FIG. 5 and FIG. 6;

FIG. 8 is a chromatogram obtained in Example 1;

FIG. 9 is a chromatogram obtained in Example 2; and

FIG. 10 is a chromatogram obtained in Example 3.

The following definitions are given for a complete understanding of the invention, and when each term described below is used herein it is meant to have the meaning given:

"Protein(s)" - biological molecules comprising a continuous chain of carbon and nitrogen atoms joined together through peptide bonds between adjacent amino acids, and all

primary, secondary, tertiary, and quaternary structures of these molecules;

"Simple proteins" - proteins which do not include a nonprotein prosthetic group, for example albumin, and transferrin;

"Complex proteins" - antibody-like protein conjugates in which at least two proteins are linked to form a biologically active complex; also, proteins which include a nonprotein prosthetic group, which may include coenzymes such as flavins and pyridine nucleotides, as well as lipids and polysaccharides;

"Antibody(ies)" - as used herein, this term is meant to include special gamma globulins (as a rule) or beta globulins (occasionally) formed within a living body against and as a result of stimulation by specific antigens. The term is meant to include "heavy" and "light" chains of antibodies, fragments of such chains, and complexes between chain fragments, chains and antibodies - includes IgM, IgA, IgG, IgD and IgE from immunoglobulins and all allotypes and idiotypes from but not limited to human, murine, goat, bovine, or rabbit species;

"Chromatographic apparatus" and "chromatographic device" - any apparatus or configuration of component elements (including conventional chromatographic columns) which separates molecules based on differing physical, chemical or structural properties;

"Chromatographic medium" and "chromatographic separation means" - as used herein, this term means the actual chromatographically active element in a chromatographic apparatus or chromatographic device, i.e., the element which affects the partitioning of molecules based on different physical, chemical or structural properties;

"pH buffer" - a liquid which "equilibrates" the pH of a chromatographic separation means or chromatographic medium;

"Hydrated crystalline hydroxylapatite particles" - hydroxylapatite which is in its substantially crystalline form, hydrated to any level of hydration, and having a particle size ranging from about 1 to about 300 microns;

"solid stationary phase" - a chromatographic medium through which passes a mobile phase;

"permanently entrapped", "permanently enmeshed", and "permanently immobilized" - as used herein, these terms refer to particles which can change their physical shape and size but not their spatial position within a porous, substantially nonswellable sheet, preventing substantial sloughing of particles and fines formation due to rubbing, and which may be physically and/or chemically held within the sheet;

"Porous, substantially nonswellable sheet" - as used herein, this term refers to a sheet of material of thickness ranging from about 0.005 inch to about 0.25 inch, which has a pore size ranging from about 0.2 microns to about 15 microns;

"Monoclonal antibodies" - as used herein, this term refers to highly specific antibodies produced by cloned cells of a single hybrid cell, the product of fusion of an antibody-producing lymphocyte and an immortal malignant antibody-producing lymphocyte cell, examples being IgM, IgG monoclonals;

"Phosphate gradient" - this term refers to eluting methods where the phosphate ion concentration is gradually lowered or raised to elute bound protein from hydroxylapatite particles.

Referring now to the drawing, the view presented in FIG. 1 shows many of the chromatography cartridge components of one embodiment in assembled form. Back pressures of 100 psi or less and 50 psi or less are preferable for all embodiments. The amount of back pressure will be substantially less than about 25 psi to maintain a constant capacity cartridge in the preferred embodiment of the invention. The assembled chromatography cartridge 25 includes an inlet nozzle 1, an outlet nozzle 2, annular containment body 3, flow distribution manifold 5, inlet seal 10, and outlet seal 15. The embodiment of the assembled chromatography column cartridge shown in FIG. 1 also includes annular volume compensation frit 30, central support or a cylindrical volume compensation frit 40, spiral-wound or core-wrapped, porous, substantially nonswellable sheet 60 containing hydrated crystalline hydroxylapatite particles 62 permanently enmeshed therein. FIG. 1 also shows the inlet flow

20, which proceeds through inlet nozzle 1 and flow distribution manifold 5 to produce a plurality of streams of solution containing antibody to be purified. Inlet seal 10 prevents inflowing sample 20 from passing through column 25 directly through central support 40. The inlet seal 10, in cooperation with the flow distribution manifold 5, forces flow to annular volume compensation frit 30.

In the preferred embodiment of the invention, annular volume compensation frit 30 is replaced by more spiral-wound or core-wrapped, porous, nonswellable composite sheet 60 and a thin layer of rigid porous frit or mesh. In both configurations, the incoming sample, after passing through flow distribution manifold 5 and into annular frit 30, contacts and completely covers the outermost surface 61 of spiral-wound or core-wrapped sheet 60. This is due to the fact that the sheet itself imparts a small differential pressure between its surfaces. After contacting the outermost surface 61 of spiral-wound or core-wrapped sheet 60, sample fluid flows through the layers of sheet 60 in a combination of axial and radially inward flows, until reaching porous central support 40, upon which said sheet 60 is wound. The sheet 60 may be attached to the porous central support 40 in methods well known in the art, although this is not required. Porous central support 40 may be made of any porous material which is inert to sample and elution fluids to be used. Preferably, the support 40 is porous polypropylene, in the preferred embodiment where no annular volumetric compensation is to be used; alternatively, porous central support 40 may be made of cellulosic depth filter material. In both cases the support 40 may serve as a residual volume compensation means. Sample fluid then flows from the porous central support to and through the outlet nozzle 2. Outlet nozzle 2 has associated with it a male Luer-lock connection 7 in the preferred embodiment, in this case a male Luer-lock with threads 8.

FIG. 2 is an enlarged cross-sectional view of one embodiment taken along the line 2-2 of FIG. 1. Chromatography cartridge 25 is shown with annular containment body 3, annular volume compensation frit 30, which is replaced by more spiral-

wound or core-wrapped sheets 60 in the preferred embodiment. Also shown in FIG. 2 is the porous central support 40.

The porous, substantially nonswellable, spiral-wound or core-wrapped sheet 60 is shown with hydrated crystalline hydroxylapatite particles 62 permanently enmeshed therein. The hydroxylapatite particle size is not critical, and can range from about 1 to about 300 microns. Preferable however, is a DNA-grade hydroxylapatite, which has an average particle size ranging from about 48 to about 52 microns, having a particle size distribution ranging from about 15 to about 60 microns. The sheets used in the present invention have a pore size ranging from about 0.2 to about 15 microns, and are wetted so that the crystalline hydroxylapatite is hydrated. The sheets are also equilibrated with a pH buffer having a pH ranging from about 5.5 to about 10 and a phosphate concentration ranging from about 0.001 to about 0.45 M.

FIG. 3 shows an exploded perspective view of all of the parts of one embodiment of a chromatographic cartridge apparatus of the present invention. Inlet nozzle 1 is connected to flow distribution manifold 5. Annular volume compensation frit 30 is shown coaxially positioned within annular containment body 3. Inlet nozzle connects at one end of body 3, while outlet nozzle 2 connects with the opposite end of annular containment body 3. Annular volume compensation frit 30, which consists of extra turns of porous, spiral-wound sheet in the preferred embodiment, is shown coaxially positioned within annular containment body 3. The spiral-wound or core-wrapped sheet 60 is positioned within the annular volume compensation frit 30. The annular compensation frit 30 may be replaced with a thin layer of rigid polypropylene. FIG. 3 further shows porous central support 40, inlet seal 10 and outlet seal 15. The seals 10 and 15 may be made of any inert nonporous material, for example, polyethylene or natural rubber.

FIG. 4 shows an enlarged cross-sectional view taken along the line 4-4 of FIG. 1. This view shows flow distribution manifold 5 and inlet seal 10. The manifold 5 and

inlet nozzle (not shown) are glued or otherwise connected to the inlet seal 10.

The inlet and outlet nozzles, annular containment means, and Luer locks may be made of any material which is inert to the sample solutions and elution solutions, and pressures and temperatures applied to the chromatography column cartridge. A preferred material is a plastic material such as polyethylene, polypropylene, or polycarbonate.

The view presented in FIG. 5 shows some of the chromatography cartridge components of a second embodiment in assembled form. The amount of back pressure will be substantially less than about 25 psi to maintain a constant capacity cartridge in the second preferred embodiment of the invention. The assembled chromatography cartridge 125 includes an inlet nozzle 101, an outlet nozzle 102, an annular containment body 103, a flow distribution manifold 104, and a flow collection manifold 105. The embodiment of the assembled chromatography cartridge shown in FIG. 5 also includes single or stacked porous, substantially nonswellable sheet 106 containing hydrated crystalline hydroxylapatite particles 107, permanently enmeshed therein.

FIG. 6 shows an exploded perspective view of all the parts of the second embodiment. The single or stacked porous, substantially nonswellable sheet is positioned between the upper part of annular containment body 126 and the lower part of annular containment body 127.

FIG. 7 is a view showing the flow channels found on the surfaces of the flow distribution manifold 104 and flow collection manifold 105.

In practicing the method of the invention, a preferable method for separating complex proteins from a sample includes:

(a) combining in a chromatographic device, in the presence of a pH buffer, a liquid containing complex proteins with a chromatographic medium comprising hydrated crystalline hydroxylapatite particles having initial surface area available to bind complex proteins, the particles permanently immobilized in a substantially nonswellable, porous sheet, the sheet

allowing the hydrated crystalline hydroxylapatite particles to interact with the liquid such that the combination of complex protein-bound surface area and unbound surface area is substantially equal to the initial surface area, thereby forming a plurality of complex protein-hydroxylapatite particle complexes; and

(b) dissociating the complex proteins from the complex protein-hydroxylapatite particle complexes by combining the complexes with at least one eluting solution flowing at about the same rate through the chromatographic medium from start to finish of the dissociating.

As used herein, the term "substantially equal to" when referring to the initial HA particle surface area means that HA fines produced are negligible. Preferably the substantially nonswellable, porous sheet is selected from the group consisting of polytetrafluoroethylene and glass fiber paper and has a format selected from the group consisting of spiral-wound core-wrapped, pleated, and stacked sheets; the buffer has a pH ranging from about 5.5 to about 10 and a phosphate concentration ranging from about 0.001 to about 0.10 M; and the sample containing proteins contains at least one class of antibody, preferably monoclonal. Those skilled in the art will recognize that "core-wrapped" includes concentric annuli of sheet material.

In this method, hydrated crystalline hydroxylapatite particles preferably comprise at least about 40 weight percent of the chromatographic medium and have a particle size distribution ranging from about 15 to about 60 microns. Particularly preferable is the case in which the hydrated crystalline hydroxylapatite particles comprise at least about 75 weight percent of said chromatographic medium and have a particle size distribution ranging from about 15 to about 60 microns.

The eluting solution comprises a phosphate gradient of increasing phosphate concentration preferably having a terminal concentration of at least about 0.14 M, more preferably at least about 0.45 M.

As used herein, the term "initial surface area available to bind" means that surface area approximated to be necessary to effect the separation, which is deemed to be within the skill of those knowledgeable in the art, typical sheet compositions being shown in the examples.

Further, the term "about the same rate" when referring to flow rate means that the flow rate decreases negligibly throughout the separation with corresponding small change in back pressure. The term "substantially no change in pressure drop" has similar meaning when used herein.

The following examples are for illustrative purposes only and are intended to neither limit nor define the apparatus and method of the invention in any manner.

EXAMPLE 1

A chromatography cartridge containing 80 square centimeters of polytetrafluoroethylene-hydrated crystalline hydroxylapatite (PTFE-HA) composite sheet comprised of 20 weight percent PTFE and 80 weight percent HA in which the HA was comprised of 72 weight percent DNA-Grade Bio-Gel HTP and 28 weight percent Bio-Gel HTP was assembled according to FIG. 1. The cartridge was connected to a gradient liquid chromatography system and equilibrated at a flow rate of 1.0 ml per minute first with 0.40 M sodium phosphate buffer, pH 6.8 (B), then with 0.01 M sodium phosphate buffer, pH 6.8 (A). The chromatography system pump was stopped and 250 microliters of a mixture of simple proteins consisting of 1 mg each bovine serum albumin, lysozyme, and equine cytochrome C in (A) was applied to the device through a three-way Luer valve using a 1 ml syringe. The pump was started and a 30 minute concave gradient from (A) to (B) was delivered to the cartridge. The cartridge effluent was monitored at an ultraviolet wave-length of 280 nanometers. The three simple proteins eluted from the cartridge within 50 minutes as shown in FIG. 8.

EXAMPLE 2

A chromatography cartridge containing five (5) layers of 25 mm circles (24.5 square centimeters) of polytetrafluoroethylene-hydrated crystalline hydroxylapatite (PTFE-HA) composite sheet comprised of 20 weight percent PTFE and 80 weight percent HA in which the HA was comprised of 72 weight percent DNA-Grade Bio-Gel HTP and 28 weight percent Bio-Gel HTP was assembled according to FIG. 6. The cartridge was connected to a gradient liquid chromatography system and equilibrated at a flow rate of 1.0 ml per minute first with 0.40 M sodium phosphate buffer, pH 6.8 (B), then with 0.01 M sodium phosphate buffer, pH 6.8 (A). The chromatography system pump was stopped and 250 microliters of mixture of simple proteins consisting of 2.5 mg bovine serum albumin, 2.0 mg chicken lysozyme, and 2.2 mg equine cytochrome C in (A) was applied to the device through a three-way Luer valve using a 1 ml syringe. The pump was started and a four-step gradient applied. After continuing briefly at 0% B, the first step was 10% B, the second 21% B, the third 32% B, and the fourth 68% B. The cartridge effluent was monitored at an ultraviolet wavelength of 280 nanometers. The three simple proteins eluted from the cartridge as shown in FIG. 9.

EXAMPLE 3

A chromatography cartridge containing five (5) layers of 25 mm circles (24.5 square centimeters) of glass fiber-hydrated crystalline hydroxylapatite (GF-HA) composite sheet comprised of 50 weight percent glass fiber and 50 weight percent HA in which the HA was comprised of 100% DNA-Grade Bio-Gel HTP was assembled according to FIG. 6. The cartridge was connected to a gradient liquid chromatography system and equilibrated at a flow rate of 1.0 ml per minute first with 0.40 M sodium phosphate buffer, pH 6.8 (B), then with 0.01 M sodium phosphate buffer, pH 6.8 (A). The chromatography system pump was stopped and 250 microliters of mixture of simple proteins consisting of 2.5 mg bovine serum albumin, 2.0 mg chicken lysozyme, and 2.2 mg equine cytochrome C in (A) was applied to the device through a three-way Luer valve using a 1

ml syringe. The pump was started and a three-step gradient applied. After continuing briefly at 0% B, the first step was 15% B, the second 34% B, and the third 68% B. The cartridge effluent was monitored at an ultraviolet wave-length of 280 nanometers. The three simple proteins eluted from the cartridge as shown in FIG. 10.

EXAMPLE 4

A chromatography cartridge containing three (3) layers of 47 mm circles (47.7 square centimeters) of polytetrafluoroethylene-hydrated crystalline hydroxylapatite (PTFE-HA) composite sheet comprised of 20 weight percent PTFE and 80 weight percent HA in which the HA was comprised of 72 weight percent DNA-Grade Bio-Gel HTP and 28 weight percent Bio-Gel HTP was assembled according to FIG. 6 and connected to the exit line of a pressure gauge. The pressure gauge was connected to a peristaltic pump and the pump connected to a reservoir of 0.01 M sodium phosphate buffer, pH 6.8. The outlet of the cartridge was connected to a calibrated flow meter to monitor the flow rate. The head pressure was approximately 15.6 PSI at 3 mls per minute, with no evidence of fines build up as would be exemplified by an increase of head pressure or decrease in flow rate.

EXAMPLE 5

The chromatography cartridge in Example 4 was connected to the exit line of a pressure gauge. The pressure gauge was connected to a peristaltic pump and the pump connected to a reservoir of 8 M urea, 0.01 M sodium phosphate buffer, pH 6.8. The outlet of the cartridge was connected to a calibrated flow meter to monitor the flow rate. The head pressure was approximately 22.1 PSI at 3 mls per minute, with similar performance as to pressure and flow as Example 4.

EXAMPLE 6

A chromatography cartridge containing three (3) layers of 47 mm circles (47.7 square centimeters) of glass fiber-hydrated crystalline hydroxylapatite (GF-HA) composite

sheet comprised of 50 weight percent GF and 50 weight percent HA in which the HA was comprised of 100 weight percent DNA-Grade Bio-Gel HTP was assembled according to FIG. 6 and connected to the exit line of a pressure gauge. The pressure gauge was connected to a peristaltic pump and the pump connected to a reservoir of 0.01 M sodium phosphate buffer, pH 6.8. The outlet of the cartridge was connected to a calibrated flow meter to monitor the flow rate. The head pressure was approximately 5 PSI at 3 mls per minute, with no substantial change in flow rate or pressure head.

EXAMPLE 7

The chromatography cartridge in Example 6 was connected to the exit line of a pressure gauge. The pressure gauge was connected to a peristaltic pump and the pump connected to a reservoir of 8 M urea, 0.01 M sodium phosphate buffer, pH 6.8. The outlet of the cartridge was connected to a calibrated flow meter to monitor the flow rate. The head pressure was approximately 5 PSI at 3 mls per minute, again with no substantial change in head pressure or flow rate.

The foregoing description is offered primarily for purposes of illustration. It will be readily apparent to those skilled in the art that further modifications, variations and the like may be introduced in the materials, configurations, arrangements and shapes of the various elements of the chromatography column structure without departing from the scope of the invention as claimed in the accompanying claims. For example, a square or rectangular cartridge could be used in the method of the invention. Other pH buffers, such as sulfate or carbonate based buffers may be developed for certain applications.

CLAIMS

1. A method of separating complex proteins from a sample comprising:

(a) combining in a chromatographic device, in the presence of a pH buffer, a liquid containing complex proteins with a chromatographic medium comprising hydrated crystalline hydroxylapatite particles having initial surface area available to bind said complex proteins, said particles permanently immobilized in a substantially nonswellable, porous sheet, said sheet allowing said hydrated crystalline hydroxylapatite particles to interact with said liquid such that the combination of complex protein-bound surface area and unbound surface area is substantially equal to said initial surface area, thereby forming a plurality of complex protein-hydroxylapatite particle complexes; and

(b) dissociating said complex proteins from said complex protein-hydroxylapatite particle complexes by combining said complexes with at least one eluting solution, said eluting solution flowing at about the same rate through said chromatographic medium from start to finish of said dissociating.

2. A method in accordance with claim 1 in which said substantially nonswellable, porous sheet is selected from the group consisting of polytetrafluoroethylene and glass fiber paper, said pH buffer has a pH ranging from about 5.5 to about 10 and a phosphate concentration ranging from about 0.001 to about 0.10 M, and wherein said proteins contain at least one class of antibody.

3. A method in accordance with claim 2 in which said at least one class of antibody consists of monoclonal antibodies.

4. A method in accordance with any of claims 1 to 3 in which said hydrated crystalline hydroxylapatite particles comprise at least about 40 weight percent of said chromatographic medium

and have a particle size distribution ranging from about 15 to about 60 microns.

5. A method in accordance with any one of claims 1 to 4 in which said at least one eluting solution comprises a phosphate gradient having a terminal concentration of at least about 0.14 M.

6. A chromatography apparatus for purifying antibodies comprising:

inlet nozzle;

flow distribution means for receiving flow from said inlet nozzle;

inlet seal;

spiral-wound or core-wrapped chromatographic separation means which is annular and having an inside and an outside cylindrical surface, comprising hydrated crystalline hydroxylapatite particles physically entrapped in a porous, substantially nonswellable sheet, said particles having initial surface area available for binding monoclonal antibodies, said sheet preventing said particles from substantially altering their spatial location within said spiral-wound or core-wrapped chromatographic separation means during purification;

porous central support means onto which said spiral-wound or core-wrapped chromatographic separation means is wrapped;

outlet seal;

outlet nozzle; and

annular containment means for axially connecting said inlet nozzle and said outlet nozzle, having an inside annular surface which does not contact said outside cylindrical surface of said spiral-wound or core-wrapped chromatographic separation means, and which contains therein said flow distribution means, said inlet and outlet seals, said porous central support means, and said chromatographic separation means.

7. An apparatus in accordance with claim 6 in which said porous, substantially nonswellable sheet is polytetrafluoroethylene.

8. An apparatus in accordance with claim 6 or claim 7 in which said central support means is porous polypropylene.

9. An apparatus in accordance with any one of claims 6 to 8 in which said hydrated crystalline hydroxylapatite particles comprise at least about 40 weight percent of said chromatographic medium and have a particle size distribution ranging from about 15 microns to about 60 microns.

10. An apparatus in accordance with any one of claims 6 to 8 in which said hydrated crystalline hydroxylapatite particles comprise at least about 75 weight percent of said chromatographic medium and have a particle size distribution ranging from about 15 to about 60 microns.

11. A method for separating complex proteins from a sample, substantially as hereinbefore described.

12. A chromatography apparatus substantially as hereinbefore described with particular reference to the drawings.

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